# Localization of Aquaporins in the Sperm Storage Tubules in the Turkey Oviduct

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**ABSTRACT** Oviductal sperm storage tubules (SST), located at the uterovaginal junction, are the primary site of sperm storage in turkeys. Sperm reside within these storage sites and may be released via a dynamic interaction between sperm mobility and a fluid current generated by the SST epithelial cells. In this study, aquaporins 2, 3, and 9 (proteins that form water channels in the plasmalemma of a variety of cells) were immunocytochemically localized within the apical aspect of the epithelial cells that form the SST. These observations support the contention that the SST epithelial cells are capable of water exchange that may interact with sperm residing within the SST.

(*Key words*: aquaporins, avian, sperm storage tubule, uterovaginal junction)

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## INTRODUCTION

Female birds can store spermatozoa for prolonged periods in the oviduct. The structural unit that actually stores the sperm is the sperm storage tubule (SST). Collectively SST are localized in the anterior 1 to 3 cm of the vagina in a region generally referred to as the uterovaginal junction (UVJ). Resident sperm are slowly released while the hen is in egg production, ascending to the infundibulum to insure an adequate population of sperm at the site of fertilization (Bakst, 1993, 1998, 2002). The mechanisms involved in sperm selection before entry into SST, and in sperm release from SST are not well characterized, although it is known that only motile and morphologically normal spermatozoa enter SST (Allen and Grigg, 1958). More recently, Froman (2003) proposed that a unidirectional fluid current would propel sperm out of the SST when sperm mobility falls below the flow rate of the fluid.

Transport of water and solutes across cell membranes is vital to all cellular function. The molecular basis for water transport across cell membranes was unknown until discovery of aquaporin (AQP)-1 by Preston et al. (1992). Ten mammalian aquaporins (AQP) have been identified. The presence of AQP in nonmammalian vertebrates, including birds, has recently been reviewed Nishimura and Fan (2002).

In general, AQP are small membrane proteins that function as water channels in a variety of tissues and cells (Verkman and Mitra, 2000). Aquaporins are essential for water homeostasis and for providing a sustained and rapid movement of water across epithelia with tight junctions (Agre et al., 1993). Aquaporins are members of the major intrinsic protein superfamily of integral membrane proteins and have been divided into 2 subgroups. The first group, the AQP, is water selective and consists of AQP-0, AQP-1, AQP-2, AQP-4, AQP-5, and AQP-6. The second subgroup, the aquaglyceroporins, is permeable to water and to other small molecules such as urea and glycerol and consists of AQP-3, AQP-7, and AQP-9.

It is not known whether AQP play a role in generating a fluid current in turkey SST. In the following study, we used immunocytochemistry to determine the presence or absence of various AQP in the SST epithelium of the turkey hen.

## MATERIALS AND METHODS

### Experimental Birds and Tissue Preparation for Microscopy

Twenty Large White turkey females,[2] 40 to 45 wk old, were used in this study. Hens were exposed to stimulatory light (14L:10D) and housed individually in cages. Feed and water were provided ad libitum. Within 1 h



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**Abbreviation Key:** AQP = aquaporins; AVT = arginine vasotocin; SST = sperm storage tubule; UVJ = uterovaginal junction.

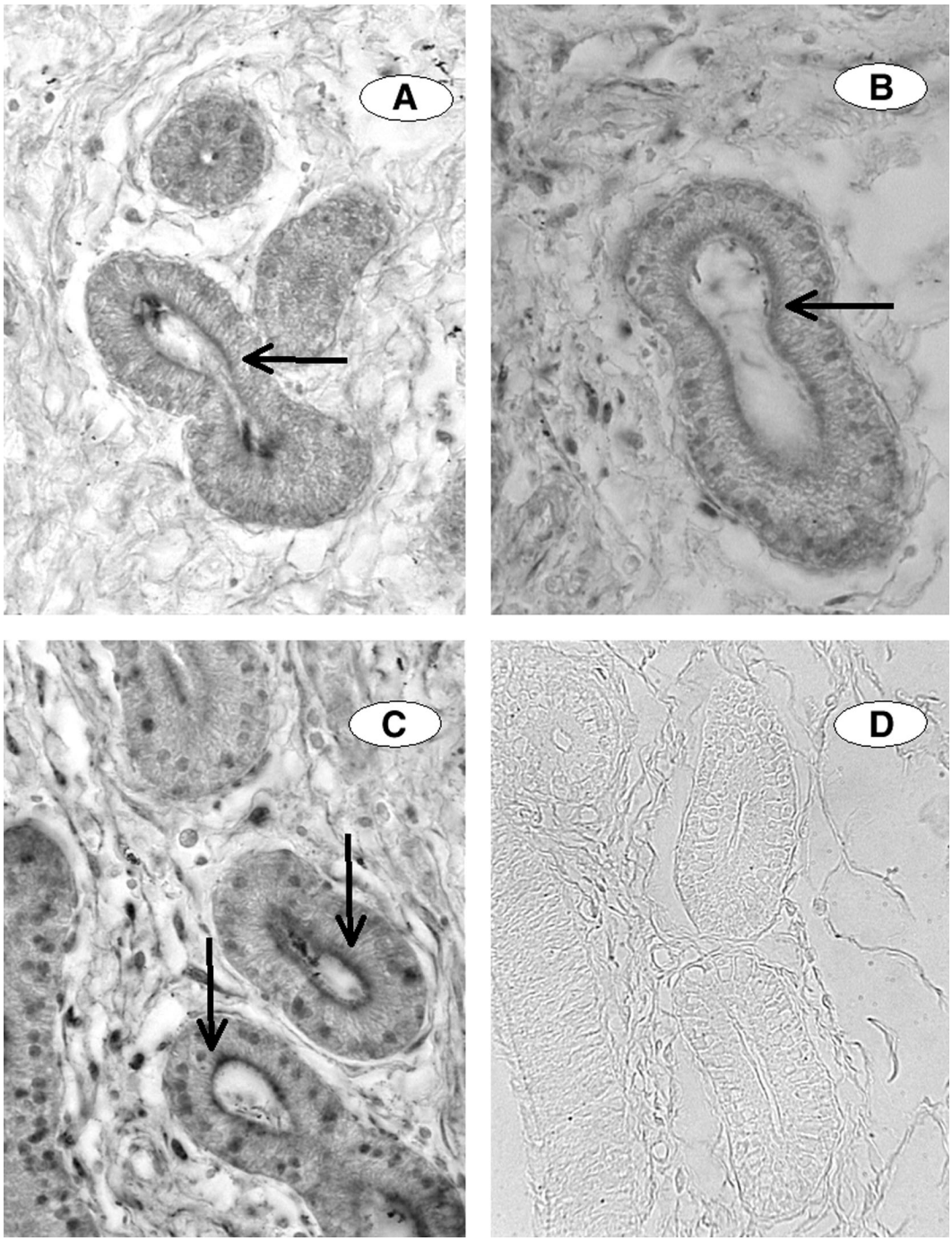


**FIGURE 1.** Panels A, B, and C: light micrographs show noncilated columnar epithelium characteristic of the sperm storage tubules (SST). Reaction product (arrows) specific for aquaporin (AQP)-2 (panel A), AQP-3 (panel B), and AQP-9 (panel C) is localized to the microvillus border and apical cytoplasm of the SST epithelial cells. Panel D: in the absence of the primary antibody, no reaction product is observed in the apical portion of the SST epithelial cells (negative control).

after laying, hens were inseminated with a single deep (4 to 6 cm) intravaginal insemination of approximately 250 million sperm and then euthanized by cervical dislocation 48 h later. The oviduct was quickly excised, and the uterus and vagina were isolated as one piece. The connective tissues surrounding the vagina and uterus were carefully removed from the underlying muscularis mucosa. After exposing the lumen, the UVJ mucosa containing SST was isolated. Small pieces (2 to 3 $mm^2$) of mucosa containing SST (verified by stereomicroscopy) were fixed overnight with PBS containing 4% paraformaldehyde[3] at 5°C. After 24 h, fixed tissues were transferred to PBS and stored at 5°C.

[3]Sigma Chemical Co., St. Louis, MO.

### *Immunoperoxidase Methods*

Samples were embedded in paraffin, and 5 mm thick sections were cut and mounted on Fisher Superfrost Plus[4] slides. Sections were deparaffinized in xylene and rehydrated in decreasing grades of ethanol solution (95, 70, and 50%). Endogenous peroxidase activity was blocked by immersion in 1.5% $H_2O_2$ in ethanol for 30 min at room temperature. Slides were rinsed with PBS and blocked overnight at 5°C with 1% milk.

Anti-rabbit-AQP antibodies for AQP 2, 3, and 9 were obtained from Alpha Diagnostic International.[5] All antibodies were diluted 1:200 in PBS containing 0.4% polyvinyl pyrolidone[3] and 1% bovine serum albumin as stabilizer, and all sections were incubated for 12 h at 5°C. After being rinsed with PBS, sections were incubated with Biotinylated Universal Secondary Antibody[6] for 2 h at room temperature then with avidin-biotinylated-peroxidase complex[7] for 30 min at room temperature. Reactivity was visualized using Vector VIP[6] and $H_2O_2$ as substrates. Sections were incubated until suitable reaction product developed (generally 15 m). After being rinsed with tap water for 5 min, sections were dehydrated in ethanol (95% and 100%) for 3 min each followed by 3 changes of xylene for 3 min each. Sections were then mounted using Permaslip[8] and examined using a Zeiss Axioskop microscope.[8]

Sections were digitally imaged using MC80 Zeiss microscope cameras[8] and Bioquant BQ-TCW98 software.[8] Specificity of immunostaining was confirmed by following the above procedures in the absence of the primary antibody (negative control).

## RESULTS AND DISCUSSION

Sperm storage tubules are a collection of tubular invaginations of the surface epithelium lining the UVJ. However, unlike this surface epithelium, which is a pseudostratified, ciliated, columnar epithelium, SST epithelium is simple columnar with a morphology and ultrastructure not suggestive of extensive secretory activity (reviewed by Bakst et al., 1994). In the present study, AQP 2, 3, and 9 gave identical positives responses primarily localized within the microvillus border and subjacent cytoplasm of the SST epithelium (Figures A, B, and C). The negative control had no reaction product (Figure D).

The presence of AQP 2, 3, and 9 in the apical portion of the SST epithelium takes on considerable significance when considering a recently proposed model of oviductal sperm storage in chickens (Froman, 2003). The foundation of Froman's theory of sperm egress from the SST is based upon the suggestion that the SST generates fluid that is released into the UVJ lumen. He explains further that this current would facilitate sperm egress from the SST when sperm mobility is less than the force of the current. It is suggested here that the presence of AQP in the apical region of the SST epithelium indirectly supports Froman's model. However, rather than Froman's implication of a constant flow through the SST lumen, we suggest that either the volume or the velocity of SST fluid secretion is modulated by factors accompanying active egg production. Indirect evidence for this hypothesis is as follows: when inseminated after the onset of photostimulation but prior to the onset of egg production, sperm retention by the SST is far greater than after the onset of egg production (Bakst et al., 1994). Maximal filling of the SST just prior to the onset of egg production implies the absence of significant fluid outflow from the SST. The onset of egg production and the accompanying cascade of cyclic endocrine hormones associated with the daily ovulatory cycle could lead to modulation of SST fluid secretion. Hypothetically, this could help synchronize oviposition/ovulation, sperm release from the SST and their transport to the site of fertilization.

Avian oviposition is partly regulated by the neurohypophysial hormone arginine vasotocin (AVT). Interestingly, circulating AVT upregulates transcription of mRNA for AQP in the collecting ducts of Japanese quail kidneys (Nishimura and Fan, 2002). The cyclic secretion of AVT, the immediate proximity of the UVJ with the uterus, and the role of AVT in the kidneys of Japanese quail leads one to speculate that fluid volume, secretion, or both may be influenced by AVT effects on SST epithelial AQP. This hypothesis and the mechanism of fluid transfer during egg plumping are the objects of future research.

To conclude, the presence of AQP 2, 3, and 9 in the SST epithelium was confirmed by immunocytochemistry. This finding is intriguing given the possibility that sperm residence within and egress from the SST may be dependent on the interaction of SST epithelial cell fluid outflow and sperm mobility (Froman, 2003).

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[5]Alpha Dignostic Intl., Inc. San Antonio, TX.
[6]Vector Laboratories Inc., Burlingame, CA.
[7]Alban Scientific Inc., St. Louis, MO.
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